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(54) A MICRO FLOW SYSTEM FOR PARTICLE SEPARATION AND ANALYSIS

MIKROFLIESSSYSTEM FÜR DIE TEILCHENANALYSE UND TRENNUNG

SYSTEME A MICRODEBIT POUR SEPARATION ET ANALYSE DE PARTICULES

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- **AHN C H ET AL: "A FULLY INTEGRATED MICROMACHINED MAGNETIC PARTICLE MANIPULATOR AND SEPARATOR" PROCEEDING OF THE WORKSHOP ON MICRO ELECTRO MECHANICAL SYSTEMS (MEM, OISO, JAN. 25 - 28, 1994, no. WORKSHOP 7, 25 January 1994, INSTITUTE OF ELECTRICAL AND ELECTRONICS ENGINEERS, pages 91-96, XP000528399**

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Description

FIELD OF THE INVENTION

[0001] The present invention relates to methods and apparatuses for detection, separation, sorting, and analysis of particles, such as cells, cell organelles, beads, molecules, such as Deoxyribonucleic acid (DNA), proteins, etc. in a fluid. In particular, the invention relates to particle separation by using different forces such as magnetic, electrophoretic, hydrodynamic and/or gravitational forces, e.g. for utilisation in flow cytometry, light microscopy, electrophoretic separation, magnetophoresis, etc.

BACKGROUND OF THE INVENTION

[0002] Flow cytometry is a well known technique that is used for high throughput measurements of optical and/or electrical characteristics of microscopic biological samples. Flow cytometry instruments analyse and isolate cells and organelles with particular physical, biochemical, and immunological properties.

[0003] Traditionally, cell sorting by flow cytometry (fluorescence activated cell sorting) has been the method of choice for isolation of specific cell populations by surface markers. However, cell sorting by flow cytometry suffers from several drawbacks, especially high dilution of desired cell sample, low speed and sterility problems. Furthermore, the equipment is very costly with high operation and maintenance cost, making the technique available only to a limited number of laboratories.

[0004] During the last few years, isolation of cells by antibody-coupled magnetic beads and carriers has been developed into a reliable tool for the isolation and characterisation of cell populations. Immunomagnetic cell separation, e.g. as commercially introduced by Dynal A/S and Miltenyi Biotec, has become an established method for cell analysis in clinical diagnostics Due to the relatively low prize, this method is attractive in flow cytometry, especially in sorting of rare cellular events. For example, sorting of fetal cells contained in maternal blood sample provides a non-invasive alternative to prenatal diagnostic procedures, such as amniocentesis of chorionic villus sampling. Another rare event scenario is the detection of low conçentration of cancer cells which has an important role in diagnosis of minimal residual disease and evaluation of appropriate therapies. Another medical application for cell sorting systems is the diagnosis of bacterial and viral diseases.

[0005] Although this method offers relatively inexpensive approach to sort rare cellular event, it adds considerable time onto the overall rare event detection and it does not offer the multiparameter analysis readily available with flow cytometry techniques. Existing techniques for magnetic separation are suffering from the low purity of the sorted cell fraction and the low recovery rate of the sorted cells. In most systems several washing steps have to be implemented into the separation procedure which then causes cell losses. Additionally small subpopulation of labelled cells cannot be directly isolated by existing magnetic separation techniques.

[0006] Separation of magnetic particles from a fluid containing the particles by means of a magnetic field is known in the art and Is disclosed e.g. in Ahn, C.H et al: "A Fully Integrated Micromachined Magnetic Particle Manipulator and Separator", Proceeding of the workshop on micro electro mechanical systems (Mem, Oiso, Jan. 25-28, 1994, no. Workshop 7, 25 January 1994, Institute of Electrical and Electronics Engineers, pages 91-96) and in US 5,053,344. No control of the transverse position of the particles in the flow channel is indicated in any of the examples and several particles may be present at the same longitudinal position at different transversal positions.

[0007] Likewise is the separation of particles from a fluid by means of an electric field known in the art as disclosed in e.g. US 4,279,345 according to which droplets are generated from the fluid containing the particles, a parameter of the particles in the droplets is observed and some of the particles are deflected by means of an electrostatic field in accordance with the observed parameter.

[0008] It is also known from US 3,827,555 to enclose a fluid containing particles in a liquid sheath so that the particles pass a detector one at a time. Particles are lead to a sort outlet instead of the ordinary outlet by means of diverting the fluid containing the particles as well as the liquid sheath to the sort outlet.

[0009] A good overview about fluorescence activated cell sorting procedures and magnetic activated cell sorting is given in Melamed et. al., "Flow Cytometry and Sorting, (Ed. Melamed et. al., Wiley & Sons Inc., 1990).

SUMMARY OF THE INVENTION

[0010] Advances in microfabrication and microfluidic technologies continue to fuel further investigation into the miniaturisation of bioanalytical instruments and biochemical assays in general. The present invention relates to development of a low cost non-invasive diagnostic test method and devices for carrying out such tests that include measuring, monitoring, sorting and analysing samples containing particles, such as organic cells, microbeads, cell organells and macromolecules such as DNA. The present invention provides a cheap, fast and reliable method and devices for

handling, sorting and analysis of such particles.

[0011] Separation may be performed according to various physical properties, such as fluorescent properties or other optical properties, magnetic properties, density, electrical properties, etc. According to an important aspect of the invention, particle separation is performed by aligning the particles in one row of particles in a micro flow channel so that particles can be treated individually.

[0012] Thus, it is an object of the present invention to provide a micro flow system and a method of particle separation having an improved efficiency of particle separation compared to the prior art.

[0013] It is another object of the present invention to provide a micro flow system and a method for particle separation in which cell lysis is minimised.

[0014] It is yet another object of the present invention to provide an improved method for preparation of fluids containing particles for separation and analysis of the particles.

[0015] It is a still further object of the present invention to provide a micro flow system and a method for simultaneous separation of particles into a plurality of groups of particles

[0016] It is a still further object of the present invention to provide a micro flow system including facilities for pre-treatment and/or post-treatment of a sample.

[0017] It is a still further objective of the invention is develop a system for separation and analysis of fetal cells in whole maternal blood samples using an integrated automated micro flow system. The system is designed by downscaling and combining different methods for handling, manipulation and analysis of biochemical samples. Thus, prenatal diagnostics by analysis of fetal cells separated from a whole maternal blood sample is an area, which can benefit from advances in miniaturisation.

[0018] It is another objective of the invention is develop a system for separation and analysis of cancer cells from a sample containing cancer cells and healthy cells using an integrated automated micro flow system. The system is also designed by downscaling and combining different methods for handling, manipulation and analysis of biochemical samples. Thus, cancer diagnostics by analysis of cancer cells separated from healthy cells is also an area which can benefit from advances in miniaturisation.

[0019] According to a first aspect of the invention the above and other objects are fulfilled by a micro flow system for separating particles, comprising a member having

a flow channel defined therein for guiding a flow of a fluid containing the particles through the flow channel,

first inlet means positioned at one end of the flow channel for entering the fluid into the flow channel,

second inlet means for entering a first guiding buffer for controlling cross-section and flow path through the flow channel of the flow of the fluid containing particles,

first outlet means positioned at the other end of the flow channel for discharging the fluid from the flow channel,

the flow of the fluid containing the particles being controlled in such a way that one particle at the time passes a cross-section of the flow channel,

the member being positioned in a field that is substantially perpendicular to a longitudinal axis of the flow channel so that particles residing in the flow channel and being susceptible to the field across the flow channel are deflected into the first guiding buffer in the direction of the field.

[0020] According to a second aspect of the invention the above and other objects are fulfilled by a method of separating particles, comprising the steps of

guiding a flow of a fluid containing the particles through a flow channel with a guiding buffer in such a way that one particle at the time passes a cross-section of the flow channel,

positioning the flow channel in a field that is substantially perpendicular to a longitudinal axis of the flow channel so that particles residing in the flow channel and being susceptible to the field across the flow channel are deflected in the direction of the field and thereby separated from the fluid containing particles and into the guiding buffer.

[0021] According to a preferred embodiment of the invention, a method of separating fetal cells from maternal cells, comprising the steps of selective magnetically staining of fetal cells in a fluid containing fetal and maternal cells, guiding a flow of the fluid containing the fetal cells through a flow channel in such a way that one fetal cell at the time passes a cross-section of the flow channel, positioning the flow channel in a magnetic field that is substantially perpendicular

to a longitudinal axis of the flow channel so that magnetically stained fetal cells residing in the flow channel are deflected in the direction of the magnetic field.

[0022] Further a method is provided for separating cancer cells from other cells, comprising the steps of selective magnetically staining of cancer cells in a fluid containing cancer and other cells, guiding a flow of the fluid containing the cancer cells through a flow channel in such a way that one cancer cell at the time passes a cross-section of the flow channel, positioning the flow channel in a magnetic field that is substantially perpendicular to a longitudinal axis of the flow channel so that magnetically stained cancer cells residing in the flow channel are deflected in the direction of the magnetic field.

[0023] The particles to be separated from other particles in a fluid and/or to be separated from the fluid containing the particles may comprise living cells, chromosomes, organelles, beads, biomolecules, such as Deoxyribonucleic acid (DNA), proteins, etc.

[0024] Preferably, the flow through the flow channel is a laminar flow so that flow of particles are predictable and easy to control, e.g. with a flow of guiding buffers.

[0025] When the flow is laminar, the stream of particles can be positioned as desired within the flow channel, e.g. by controlling flow velocities of the fluid containing particles at the particle inlet of the member and flow velocities of guiding buffers at corresponding inlets.

[0026] Preferably, the flow channel is small for the flow through the channel to have a low Reynolds number, e.g., in the range of 0.01-500, such as 0.05-50, preferably 0.1-25. Thereby, inertial effects, which causes turbulence and secondary flows are negligible, viscous effects dominate the dynamics, and mixing is caused only by diffusion. Flow of the sample, which is the fluid containing particles and guiding buffers can be laminated in guided layers through the channel and displacement of particles in the channel is only caused by the external force applied. The Reynolds number referred to is based on the hydraulic diameter of the flow channel, the flow velocity in the axial direction and the fluid density and viscosity, $Re=\rho Dh/\mu$ where the hydraulic diameter Dh is defined as four times the cross-sectional area divided by the wetted perimeter.

[0027] The illustrated flow channels of the micro flow system have a width ranging from 0,1 to 0.65 mm, preferably ranging from 0.1 to 0.4 mm, in particular ranging from 0.1 to 0.2 mm, and a depth ranging from 0.04 to 0.2 mm, preferably ranging from 0.04 to 0.1. With respect to the lowest cross-sectional area of the flow channel, it is preferred that this area is in the range of 0.004 to 0.11 mm^2, in particular in the range of 0.004 to 0.02 mm^2.

[0028] It is believed that any length of the flow channel within the range of 0.1 to 20 mm, preferably 1.0 to 3.5 mm, would lead to satisfactory results.

[0029] Preferably, the system is operating with total volumetric flow rates of 0.1 up to 200 µl/min, which gives a flow velocity of 15 mm/min up to 180 mm/min. The average residence time of a particle inside the flow channel, which corresponds to a separation time ranging from 0.1 to 6 sec. The residence time of the sample is defined by the total volumetric flow rate of the system.

[0030] The micro flow system may comprise flow rate adjustment means for adjustment of the time the particles reside in the flow channel.

[0031] Preferably, the fluid channel is sized so that for efficient separation, particles are displaced 10 - 30 µm in the flow channel. Thereby, the particle may only be exposed to a field for a very short period of time and thus, continuous separation of particles may be facilitated.

[0032] In order to collect the particles, which are deflected in the flow channel, the micro flow system may further comprise second outlet means for discharging particles having been deflected in the flow channel.

[0033] The micro flow system comprises second inlet means for entering a first guiding buffer into the flow channel together with the fluid containing particles. When the flow is laminar, the two fluids flow through the flow channel in parallel abutting each other along a small area extending along a longitudinal axis of the flow channel whereby the cross-section and the path through the flow channel of the flow of the fluid containing particles may be controlled by the first guiding buffer flow. Further, particles in the fluid containing particles may be deflected into the guiding buffer fluid when the two fluids pass the field essentially perpendicular to the longitudinal axis of the flow channel. Furthermore, two (or even more) outlets may be provided at the down stream end of the flow channel for discharging the guiding buffer now containing separated particles and fluid substantially without particles susceptible to the field essentially perpendicular the flow channel, correspondingly.

[0034] The micro flow system may further comprise third inlet means for entering a second guiding buffer for improved control of the path of particle flow through the flow channel. By adjustment of the flow velocities of the guiding buffers and the fluid containing particles, the flow within the flow channel of fluid containing particles may be controlled to flow within an essentially cylindrical shaped domain with a longitudinal axis extending substantially parallel to a longitudinal axis of the flow channel and further the position within the flow channel and the diameter of the flow cylinder may be controlled by corresponding adjustments of the volumetric ratio between the flow rate of the fluid containing particles and the flow rate of the guiding buffers.

[0035] It is possible to control the cross-sectional area of the domain containing the sample to be a little larger than

the cross-sectional area of the particles by adjusting the volumetric flow rates of the sample and of the one or two guiding buffers in such a way that the particles contained in the sample are aligned in a single row of particles. This is a very important feature since it enables individual treatment of each particle and it leads to a sensitive method of sorting particles according to their susceptibility to a field. A sample flow layer thickness less than 1 μm may be achieved.

[0036] Preferably, the channel depth is small enough, e.g. below 50 μm, to allow observation of the particles flowing through the channel by a microscope. In an important embodiment of the present invention, the micro flow system comprises a cover, e.g. a transparent or translucent cover, for covering the flow channel. When the cover is transparent or translucent, it will be possible to observe events in the flow channel, e.g. passage of a stained or coloured particle or cell.

[0037] The member with the flow channel may be produced from any suitable material, such as silicon, polymers, such as Plexiglas, Teflon, etc., glass, ceramics, metals, such as copper, alumna, stainless steel, etc., etc.

[0038] The channel may be provided in the member by any suitable manufacturing process, such as milling, etching, etc.

[0039] In a preferred embodiment of the invention, the member is a silicon chip manufactured utilising photolithography and the channel is etched into the silicon chip.

[0040] The field may be a magnetic field, an electric field, a gravity field, etc., and any combination of such fields.

[0041] A magnetic field may be generated by permanent magnets, such as rare earth magnets, such as samarium-germanium magnets, a mixture of ferromagnetic powder and epoxy, etc., etc., electromagnets, e.g., in silicon integrated electromagnets, etc. The magnets are preferably positioned adjacent to the flow channel so that the magnetic field is substantially perpendicular to a longitudinal axis of the flow channel.

[0042] In a preferred embodiment of the invention, the magnets are positioned in and glued to rectangular slots that are etched into a silicon chip. The slots are located adjacent to the separation flow channel. In the example shown in Fig. 1, a permanent magnet or an electromagnet can be received by slots in the micro flow system. The slots are, e.g., 0.5 mm wide, 0.5 mm long and 0.2 mm deep. For generation of a magnetic field, a solid magnetic block, i.e. rare earth magnet can be glued into the slot. Alternatively, a mixture of ferromagnetic powder and epoxy can be injected into the slots to produce a high magnetic field gradient.

[0043] The strength of the magnetic field inside the micro flow system may be adjustable. If an electromagnet is used for generation of the magnetic field, the magnitude of the field may be varied by varying the amplitude of the voltage input to the electromagnet. If a permanent magnet generated the magnetic field, the magnitude of the field may be varied by varying the distance between the magnet and the flow channel of the micro flow system.

[0044] As already mentioned, the net displacement of a particle in the micro flow system depends on the force applied to it by the field. This can be utilised for separation of a first group of particles of various types in a fluid into a plurality of set of particles; each set comprising a specific type of particles. A micro flow system with e.g. five separation outlets may be used to separate a fluid containing particles into five sets of particles, each set comprising particles that are influenced by the field with a force of a specific magnitude, in the following denoted particles with a specific F-value. Particles with a low F-valuc are only deflected by a small amount by the field and are discharged from the flow channel through a corresponding outlet port. Particle deflection is increased with increasing F-values whereby such particles are discharged from the flow channel through the corresponding other outlets.

[0045] The particles to be separated from other particles in a fluid and/or to be separated from the fluid containing the particles may be magnetically stained to facilitate separation in a magnetic field.

[0046] In the present context, the term staining is to be understood in a broad sense. The term is intended to cover any way of marking a particle thereby facilitating detection of the particle. For example a cell may be stained with a fluorescent substance, such as acridin orange, methylene blue, etc, facilitating detection of the stained particles by a fluorescence detector, or, a particle is said to be magnetically stained when it is coupled to a magnetic microbead. The microbead may for example carry a monoclonal or polyclonal antibody on its surface for coupling to an antigene of a cell to be separated utilizing a magnetic field.

[0047] In the case where particles have to be detected in a flow channel by optical means, such particles are preferably stained with a chromophoric reagent, or, a fluorescent probe.

[0048] An electric field may be generated by electrodes, such as metal electrodes, such as gold electrodes, etc. The electrode may be positioned inside the flow channel, e.g. to introduce electrophoretic forces, e.g. for separation of charged molecules in the fluid, or outside the flow channel e.g. to introduce dielectrophoretic forces, e.g. for separation of particles contained in the flow according to the susceptibility of the particles to the field. Preferably, the electrodes are positioned in such a way that the electric field is essentially perpendicular to a longitudinal axis of the flow channel.

[0049] The electric field may be a high frequency field, e.g. a 5 MHz field generated by electrodes positioned inside the flow channel. Living cells positioned in an electric field will be polarized and will be influenced by the field and thus, an alternating field may be used to separate living cells from other particles.

[0050] The field generated across the flow channel may be utilised for immobilisation of particles whereby particles may be held in substantially fixed positions within the flow channel for a specific period, e.g. as outlined in Fig. 6,

allowing chemical reactions with the particles to take place and/or kinetic measurements on the particles to be performed and/or to bring the particles into contact with different chemical substances or for separating the particles from the sample. The particles may undergo a washing step before removal or reduction of the field redisperses them.

[0051] According to another embodiment of the invention, the flow through the sort outlet is not continuous but only allowed by a controlling means, e.g. a valve, when a particle with the desired characteristics is detected by a detection means. The particles are sorted using hydrodynamic forces in the sense that the flow is diverged from the ordinary outlet to the sort outlet only when it contains a particle that fulfils certain criteria. The concentration of sorted particles in the flow out of the sort outlet will consequently be high. This is especially an advantage for sample flow with rare occurrence of particles that are searched for. The detection means can be e.g. optical detection means or magnetic detection means e.g. a Hall sensor or means for detecting e.g. electrical or other properties of the particles. The detection means can in an alternative embodiment be used for counting of particles with the desired characteristics as a separate function or in connection with any of the other embodiments described herein.

[0052] In yet another embodiment, the field strength is adjustable, e.g. by adjusting the voltage supplied to an electromagnet or to a set of electrodes or by adjusting the distance from a permanent magnet to the flow channel. Particles are in a first operation mode entrapped inside the flow channel by the field at high intensity while at the same time the sort outlet is closed. In a second operation mode, the field is reduced and the sort outlet is open in such a way that the entrapped particles are redispersed and moved out of the sort outlet. Particles that are rare in the sample can by switching between these two operational modes be sorted out in a highly concentrated form. An example of this embodiment is outlined in Fig. 6.

[0053] In a further interesting embodiment, the micro flow system according to the invention involves facilities for performing pre-treatment and/or post-treatment of the fluid comprising the particles. These possibilities are outlined in Figs. 5(f), 7 and 10. As an example, the particles may be treated with a reagent before entering the flow channel, e.g. undergo magnetic or chromophoric staining. Post-treatment may comprise means for collecting or concentrating the deflected particles or means for contacting the deflected particles with one or more reagent(s).

[0054] By one possible combination of the pre-treatment and the post-treatment facilities, cells may undergo magnetic staining before entering the flow channel, and after separation the staining may be removed by treatment of the stained cells with a suitable reagent.

[0055] It is an important advantage of the present invention that a micro flow system is provided that operates continuously with no requirement for operator intervention.

[0056] It is another advantage of the present invention that separation may be performed in one step.

[0057] It is still another advantage of the present invention that the particles can be separated in a continuous flow without substantially interfering with the flow itself and that separated particles may be collected at corresponding separated outlets of the flow channel without having to interrupt the flow in the flow channel.

[0058] It is another important advantage of the invention that the particles contained in the sample by the adjustment of the flow rate of one or more guiding buffers can be lined up in one row such that the particles can be analysed and sorted individually. This results in a sorting system with the highest sensitivity to the susceptibility of the single particle to the field applied to the sorting channel and a sorting system with the highest resolution of the detection means of the characteristics exhibited by the particles.

[0059] It is yet another advantage of the present invention that the micro flow system is easily integrated into other continuous flow systems, such as flow cytometers, flow injection analysis systems, etc.

[0060] It is a further advantage of the present invention that particles may be separated into a plurality of groups of particles, e.g. different subpopulations of cells, based on different susceptibility to the field generated across the flow channel of the different groups of particles. This may be obtained by using a multiple outlet micro flow system as outlined in Fig. 5(c).

[0061] It is a still further advantage of the present invention that the micro flow system allows observation of particles in the flow channel using a microscope.

[0062] It is a still further advantage of the invention that a closed system is provided allowing biohazardous samples, such as samples containing pathogens, to be entered into the system without contaminating the laboratory environment and without causing hazard for operators working with pathogen biomaterials.

[0063] It is a still further advantage of the invention that a system with a low shear stress in the flow is provided allowing a gentle treatment of biological samples; e.g. fragile living cells, especially when two guiding buffers are introduced in the channel.

[0064] It is a still further advantage of the invention that a high concentration of the sorted particles can be obtained even from samples with rare occurrence of particles that are searched and sorted for.

[0065] According to an important aspect of the invention, a new system for immunomagnetic cell separation and manipulation is provided that utilises a silicon based micro fabricated flow chip. The system combines the advantage of flow cytometry and immunomagnetic separation technique. The flow chip will be an important component of a portable micro system for cell sorting and analysis. The flow chip is designed for rapid immunomagnetic cell separation nearly

without any pressure drop. Its simple and cheap fabrication and versatile sorting and detection properties offer an alternative to conventional cell separation systems.

[0066] It is an advantage of the invention that a micro flow system is provided that is cheap, easy to operate, versatile, simple and portable and that offers the possibility of automation.

[0067] A miniaturised flow channel system is provided that utilises the advantageous fluid behaviour in micro systems. The invented system operates continuously. Instead of holding back the magnetisable particles in the separation unit, the particles are deflected into the direction of the magnetic field while passing it continuously. By splitting the fluid flow into two or more outlets, the deflection of the particles can be used for separation of particles into different sets of particles, each of which leaves the flow channel through a specific outlet.

[0068] The continuous separation system (CSS) allows efficient enrichment as well as depletion of lahelled sample contents of interest. The CSS is designed to fit under a microscope allowing parallel detection of the optical properties of the sample and the control of separation of particles.

[0069] An advantage of the geometry of the invented separation flow channel is that a magnetised or electrically charged particle has to be moved only over a distance of 10 - 30 μm to be separated from the fluid containing particles.

[0070] Furthermore, the invention enables isolation of multiple cell or particle subpopulations from a single sample at the same time. The magnitude and direction of the force F on a magnetisable particle, e.g. a magnetically labelled cell, is dependent on the magnitude of the magnetic field and the number of magnetic moments inducible on a labelled cell.

[0071] $F = N{*}S{*}\mu B{*}\,\mathrm{grad}\,B$

where S is the number of Bohr magnetons (μB) per particle and N is the number of particles per cell.

[0072] Beads with small S are moving a less distance in lateral direction in relation to the flow through the flow channel than beads with a higher S value. This can be used to separate subpopulation of cells labelled with different magnetisable beads: By splitting the flow channel in various outlet channels cells can be distinguish and separated due to their individual F values.

[0073] The drag force on a spherical particle can be found from the particle Reynolds number, based on particle diameter, particle velocity relative to the fluid and fluid viscosity and density. In a flow with a Reynolds number less than 100, the drag force D on the particle can be found from a modified version of Stokes law

$$D = 3\pi\mu\, Ud(1+3/16Re)^{\frac{1}{2}}$$

where μ denotes the viscosity of the liquid, U is the relative velocity of the particle and d is the diameter. The numerical value of the parenthesis on the right hand side of the above formula is close to unity for Reynolds numbers less than one why it in that case can be omitted. The magnitude of the drag force on the particles, the force applied to the particle by the field, the distance the particle needs to be moved and the time available for the separation are all important aspects to be considered when a separation process and the device for carrying it out is designed.

[0074] An example is given for separation by gravitational means. The effective gravitational force G defined as the gravitational force minus the buoyancy force is found as

$$G = (\rho_{particle} - \rho_{liquid})g\,\frac{\pi}{6}d^3$$

where g is the gravitational constant. For simplicity, a Reynolds number for the particle of less than one is assumed why the drag force D is given in a simple form. These two forces, D and G, are equal when the maximum velocity, the settling velocity U_∞ has been reached. This velocity is found as

$$U_\infty = \frac{(\rho_{particle} - \rho_{liquid})g\,d^2}{18\mu}$$

[0075] The velocity to a given time t can be found as

$$U(t) = U_\infty(l - e^{-t\frac{g}{U_\infty}})$$

[0076] For a particle submerged in water with a diameter of 30 μm and a density of 1.2 times the density of water the settling velocity is 9×10^{-5} m/s. The particle will reach 90 % of this velocity after 2.1×10^{-5} seconds why the transient phase can be neglected. It will take the particle 0.33 seconds to travel a distance of 30μm, which makes the method reasonable to employ for separation purposes.

[0077] While instrumentation in chemistry and biochemistry has become more automated in recent years, the preparation of samples remains a highly laboratory intensive task. The demand is increasing for high throughput, easier to use cost effective analytical devices and assays. Creating this opportunity is e.g. the world-wide effort to sequence the Human Genome, resulting in the development of new DNA diagnostics and therapeutics. Another important trend is the minimisation of health care costs and hospital admissions by testing patients and monitoring therapeutic use in less expensive environments, the so-called point-of-care analysis.

[0078] Micro flow devices containing arrays of nucleic acid hybridisation, sites, known as genosensors, are being developed for a variety of uses in genomic analysis. A great deal of the overall genosensor development effort involves optimisation of experimental conditions in the actual use of genosensors.

BRIEF DESCRIPTION OF THE DRAWINGS

[0079] Exemplary embodiments of the invention will now be described with reference to the accompanying drawings in which

Fig. 1 illustrates the operation of particle separation according to the present invention,

Fig. 2 shows a cross-sectional view of a separation flow channel according to the present invention. (a) shows the main embodiment and (b) shows a cross-sectional view of a separation flow channel for gravitational separation,

Fig. 3 shows a micro flow system with electrodes as field generating means,

Fig. 4 shows a flow diagram of a magnetic particle separation apparatus according to the present invention,

Fig. 5 shows flow diagrams of various embodiments of the present invention. (a)-(d) show embodiments with various numbers of inlets and outlets, and (e) shows an embodiment with an enlarged separation chamber, and (f) shows an embodiment with an enlarged chamber for collecting separated particles,

Fig. 6 illustrates entrapment of magnetic particles in a flow channel,

Fig.7 shows a flow diagram of two flow channels coupled in parallel (a) and in sequence (b) and (c),

Fig. 8 illustrates the principle of introducing a pre-treatment facility in the member comprising the micro flow system, here further combined with a post-treatment facility or a hydrodynamic separation facility,

Fig. 9 illustrates the preparation of a micro flow system,

Fig. 10 shows diagrams from the magnetic separation described in Example 3, and

Fig. 11 is a flow chart illustrating a process for separating fetal cells from a maternal blood sample by combining different separation methods as described in Example 4.

DETAILED DESCRIPTION OF THE DRAWINGS

[0080] According to a preferred embodiment of the invention, magnetically stained particles, e.g. cells labelled immunologically with magnetic particles, such as antibody-coupled magnetic beads, are separated from non-magnetic particles, i.e. non-labelled cells, by exposing the particles to a magnetic field generated with a permanent or an electromagnet. Positive or negative selection methods may be employed. By positive cell separation, cells of a specific cell type are separated and isolated from a heterogeneous mixture of cells.

[0081] Fig. 1 illustrates the principle of the separation method according to the invention. A micro flow system 1 is shown having three inlet and two outlet ports. The sample 9 containing particles enters the separation flow channel 5 through a central inlet port 2 and is continuously guided through the separation flow channel 5 of the micro flow system 1 by two guiding buffers 10 and 11, each of which enters the separation flow channel through inlet ports 3 and 4, respectively. A field generating means comprising a magnet 8 is located adjacent to the flow channel 5 and generates

a magnetic field across the flow channel 5. When the sample 9 containing particles passes the magnetic field, magnetically stained particles 12 are drawn into the guiding buffer 10 and leave the flow channel 5 together with the guiding buffer 10 through the sort outlet 6 while non-labelled cells 13 which are not influenced by the magnetic force remain in the fluid 9 leaving the flow channel 5 through the waste outlet 7.

[0082] Due to the small channel dimensions, the flow is laminar with negligible influence of inertial forces. Mixing of the sample flow and the guiding buffers is not detectable since the contact area is small and the contact time is reduced to a subsecond range. The thickness of the sample flow can be precisely adjusted by variation of the flow rate of the two guiding buffers. This enables the adjustment and optimisation of the magnetic micro flow system for various cell types and sizes. The volume flow of the sample and the two guiding buffers are adjusted so that the particles in the sample are lined up into a single stream of particles.

[0083] The magnetic field in the micro flow channel operates as an extremely sensitive filter for magnetic particles, e.g. cells. Cells labelled with superparamagnetic beads (e.g. MACS, Dynal) are magnetised and attracted by the magnetic field whereby the flow of magnetised particles is deflected into the sort outlet. The short residence time of the fluids in the flow channel and the low Reynolds numbers of the flow in the flow channel minimise the influence of gravity compared to the influence of the magnetic force.

[0084] Fig. 2 shows a cross-sectional view of two variants of the micro flow system 1 manufactured utilising semiconductor technology. The micro flow system may be manufactured in any suitable material such as polymers, glass, semiconductors, such as silicium, germanium, gallium arsenate, etc., etc.

[0085] The first micro flow system (a) shown is a 3-layer sandwich. The central layer 14 is a silicon wafer having a flow channel 5 etched into it. The silicon wafer 14 is covered with a transparent plate 15, such as a glass plate, having a thickness of, e.g., 0.16 ma. Fluids inside the flow channel 5 may be observed through the glass plate 15, e.g. utilising a microscope 16 (detection means). The fluid inlet 2 and outlet 7 are connected to tubings 17, 18, e.g. fused silica capillary or Teflon tubings, for entering fluids into or discharging fluids from the flow channel 5. Buffer inlets 3 and 4 and the outlet 6 for the separated particles are not shown. The bottom plate 19, e.g. made of plastic, facilitates mounting of the tubings 17, 18.

[0086] A modified version (b) of the micro channel system for separation was designed with gravitation as the force field, thus sorting particles due to their density and/or diffusion constant, the latter mainly being controlled by the shape and size of the particles. The system is during operation positioned with the flow plane substantially perpendicular to the direction of the force of gravity. As illustrated in Fig. 2(b), this embodiment of a micro flow system 1 has a sample inlet port 2 and an outlet port 7 located above the micro channel 5 and a buffer inlet port 3 and an outlet port 6 located below the micro channel 5. The sample containing particles 9 enters the separation flow channel through inlet port 2, and a guiding buffer 10 enters the separation flow channel 5 through inlet port 3. In this way, two laminated layers of fluid extending along the horizontal plane are created continuously flowing through the separation flow channel 5 of the micro flow system 1. Particles move from the particle containing layer to the guiding buffer layer by sedimentation. When the sample containing particles 9 passes the flow channel 5, particles with certain density and size properties are drawn into the guiding buffer 10 by the gravitational force and leave the flow channel 5 together with the guiding buffer 10 through the outlet port 6 while particles which are less susceptible to the gravitational field remain in the sample 9 leaving the flow channel 5 through the waste outlet 7. The vertical displacement of a specific particle in the sample is given by its density and diffusion constant and the contact time of the sample layer with the guiding buffer layer. The contact time is defined by the total flow rate of the fluids passing through the micro systems 1 and the length of the micro channel 5. The system can be adjusted such that a desirable or appropriate specimen can be withdrawn and separated from the sample flow due to their density and/or diffusion properties by adjusting the volumetric flow rates of the guiding buffer and particle containing sample.

[0087] Alternatively, the micro flow system may comprise two further inlet ports for entering a second and a third guiding buffer into the micro channel 5, where the two further inlet ports are positioned above the micro channel, one on each side of the sample inlet port 2. The flow rates of the sample and the second and third guiding buffers may be adjusted so that the particles contained in the sample are lined up in a single line.

[0088] Characteristic features of an exemplary embodiment of a micro flow system according to the invention, e.g. as shown in Figs. 1 and 2, is shown in Table 1.

Table 1

Characteristics, micro flow system	
Manufacturing method	Material: Silicium Oxide, SiO_2 Photo-lithography Wet-chemical etching
Flow Channel	
Cross sectional area Length	0.1 - 0.55 mm width x 0.04-0.2 mm depth 1.0 - 200 mm

Table 1 (continued)

Characteristics, micro flow system	
Manufacturing method	Material: Silicium Oxide, SiO_2 Photo-lithography Wet-chemical etching
Flow Channel	
Total flow rate [µl/min] Flow velocity [mm/min] Reynolds number Separation time	1 - 200 15 - 180 0.1 - 20 0.1 sec - 6.0 sec [2 µl/min]
Magnet	
Permanent Magnet	
Rare Earth Samarium-Germanium 0.5 x 0.5 x 0.2 mm	
Electromagnet	
Holding Magnet 25 mm 12 V D.C. RS	

[0089] Fig. 3 shows a micro flow system 1 utilising electrodes 20, 21 to generate an electric field across the flow channel 5. The electrodes 20, 21 may introduce dielectrophoretic or electrophoretic forces utilised for particle separation. For electrophoretic separation to take place, gold electrodes may be positioned at the inside of the walls of the flow channel 5. By applying a voltage across the electrodes, an electrical field is generated substantially perpendicular to a longitudinal axis of the flow channel. The electrical field cause deflection of charged particles or molecules in the flow channel 5 whereby electrically charged particles can be deflected away from the sample containing particles flowing in the micro flow channel and into a guiding buffer also flowing in the flow channel and abutting the sample containing particles in the micro flow channel.

[0090] Fig. 4 shows a micro flow apparatus 22 including a micro flow system 1 as shown in Figs. 1 and 2. The micro flow system 1 has two inlets 2, 3 and two outlets 6,7, two syringe pumps 23, 24, two 3way control valves 25,26 and capillary tubings 27, 28. The capillary tubings 27, 28 are used for interconnecting the two syringe pumps 23, 24 with the inlets 3, 2, respectively, of the micro flow system 1.

[0091] Conventional syringe pumps with means, e.g. stepping-motors (not shown), to move the pistons at a predetermined speed have been utilised for generating a continuous flow of the guiding buffer through the inlet tube 27 and a continuous flow of the sample through the inlet tube 28. The system can be operated in a first loading mode where the two 3-way control valves 25, 26 open for flow between the syringe pumps 23, 24 and the buffer reservoir 29 and the sample reservoir 30, respectively, and the syringe pumps 23, 24 are loaded with buffer and sample from the reservoirs 29, 30, respectively. In a consecutive second operational mode the two 3-way valves 25, 26 open for flow between the syringe pumps 23, 24 and the capillary tubing 27 to the buffer inlet 3 and the capillary tubing 28 to the sample inlet 2 of the micro flow system 1, respectively. The syringe pumps are in this second operational mode controlled to generate a predetermined volumetric flow rate through the micro flow system 1.

[0092] Fig. 5 illustrates various micro flow systems 31, 32, 33, 34, 35, and 36 having flow channels of different geometries, illustrating different embodiments of the invention. Micro flow systems with two or three inlet ports and two, three or five outlet ports, respectively, are shown in Figs. 5(a)-(d). The system shown in 5(a) with inlet ports for sample and two guiding buffers, respectively, and sort outlet port and waste outlet port is similar to the system shown in Fig. 1. Figs. 5(b) and (c) show systems with multiple outlet ports, three and five, respectively, whereto particles can be sorted and leave the flow channel through according to their susceptibility to the applied field. A simple system with two inlet and two outlet ports are shown in Fig. 5(d) similar to the one in Fig. 2(b) that is used for gravitational sorting. A micro flow system with a separation channel equipped with a magnet where the width of the separation channel is enlarged before the bifurcation in a sort outlet and a waste outlet is shown in Fig. 5(e). According to the behaviour of liquids in a flow channel, the size of the cross-sectional area occupied by the sample flow is proportional to the width of the separation channel. According to this, the transversal distance between two particles A and B is increased proportional to the increase of the width of the separation channel. A larger distance between particles, which are to be separated, yields a higher selectivity of the mechanical separation. Fig. 5(f) shows a micro flow system where the width of the outlet channel 6 is increased to form a chamber where the sorted particles are collected for further processing or analysis, e.g. detection, staining, destaining or cultivation.

[0093] Fig. 6 illustrates a system in which particles are entrapped inside the micro flow channel 5 for a desired period using the electromagnet-equipped apparatus. In this case, the magnetic field is adjusted so that magnetic particles 12 are drawn to the inner wall of the micro flow channel 5 close to the electromagnet 8. Upon removal of the current to

the electromagnet 8 the particles 12 are redispersed and are rapidly moved to the sorting outlet port 6. This 2-step sorting procedure is an alternative to the continuous sorting procedure that is particularly useful in sorting of extremely rare events where dilution of the sorted cell fraction could be a problem. The sorting outlet port 6 may be closed when the current to the electromagnet 8 is turned on and is open when the current to the electromagnet 8 is turned off. The figure shows magnetic particles 12 in the process of being withdrawn from a continuous sample flow 9. The magnetic particles 12 are attracted by the magnetic field and withdrawn from the sample flow 9 by precipitation at the inner wall of the micro flow channel 5 proximate to the electromagnet 8. When the current supplied to the electromagnet 8 is turned off, the magnetic particles 12 are released into the flow again. The separation flow channel may not have a sort outlet, instead a buffer may enter the micro flow channel 5 after the sample and the entrapped particles may be released by removing the current supplied to the electromagnet 8.

[0094] Fig. 7(a) shows two flow channels 45, 46 operating in parallel. The sample containing particles enters the flow channels 45, 46 through inlet ports 47, 48, respectively. The guiding buffer enters the flow channels through the inlet ports 49, 50, respectively. In the flow channels 45, 46, particles susceptible to the magnetic field generated by magnets 51, 52, respectively, are deflected from the sample containing particles into the corresponding guiding buffer and flow thereafter through the sort outlet 53. The remaining part of the sample leave the flow channels 45, 46 through the waste outlets 54, 55, respectively. Separation is increased by using a plurality of flow channels coupled in parallel.

[0095] Fig. 7(b) and (c) shows examples of combinations of micro flow systems for magnetic, hydrodynamic or gravitational separation. In Fig. 7(b), particles are first separated from a sample in a magnetic separation channel, where after the sorted particles are subjected to a hydrodynamic separation due to the optical properties of the particles. Thus, it is possible to analyse and separate particles from a sample based on both optical and magnetic properties of the particles or to another combination of properties or characteristics. In Fig. 7(c), two magnetic separation channels are coupled in series in order to obtain a highly purified product.

[0096] Fig. 8 illustrates an example of a micro flow system having means for automated labelling of particles with fluorescence or magnetic probes. The system may be combined with post-treatment means for removal of the probes or for other treatment of the sorted particles. The system contains a micro flow system containing a channel 57 for addition of liquids to the sample, e.g. reagents for cell lysis or staining, a channel 58 for incubation and cultivation or storage of the sample for further processing and a separation channel 5. A sample is introduced into the micro flow system via an inlet 2 and one or more reagents can be added continuously to the sample, which is transported into the incubation channel 58. A simple micro flow structure was constructed for sample pre-treatment. Preferably, the flow rates are managed by computer-controlled syringe pumps. The incubation period between mixing and analysis of the sample is given by the volumetric flow rate of the syringe pumps and the cross-sectional area and length of the incubation channel.

[0097] Fig. 9 shows a micro flow system manufactured as a 3-layer sandwich. The central layer is a silicon wafer having a flow channel etched into it. The silicon wafer is covered with a transparent plate, such as a glass plate, having a thickness of, e.g., 0.16 mm. Fluids inside the flow channel may be monitored through the glass plate, e.g. utilising a microscope or other optical detection means. The fluid inlet and outlet are connected to tubings, e.g. fused silica capillary or Teflon tubings, for entering fluids into or discharging fluids from the flow channel. Buffer inlets and the outlet for the sorted particles are not shown. The bottom plate, e.g. made of plastic, facilitates mounting of the tubings.

[0098] Figs. 9(1) to (5) illustrates the following description of the manufacturing and preparation of a micro flow system. A separation flow channel was designed to fit into a system comprising a bonded silicon/glass sandwich. The micro channels were etched into a silicon wafer covered with a boron glass plate having a thickness of 0.2 mm allowing monitoring of the micro channels, using i.e. a microscope. The separation flow channel was fabricated on a 4", 350μm, <100> silicon wafer. A 1.5μm layer of SiO_2 was applied to the surface of the silicon wafer and was patterned with a mask containing the channel layout. A 2.6μm layer of photoresist was spun on top of the SiO_2 and patterned with a mask defining intermediate holes. The two-step mask consisting of a SiO_2 mask and a photoresist mask was used for etching a two level structure with vertical walls by reactive ion etching (RIE) in a SF_6:O_2 plasma. The holes were initially etched to a depth of 22μm and then etched deeper together with the channels, which were etched to depths in the range from 40μm to 100μm A layer of 1.8μm SiO_2 was patterned with a mask for inlets and outlets on the back of the silicon wafer. The etching was carried out in KOH at 80°C and was stopped when all the intermediate holes were clearly visible from the back. Finally, a glass wafer was anodically bonded to the silicon wafer. The micro channels were designed for volumetric flow rates of 0.1 to 200 μl/min with a mean flow speed of maxmum 100 (mm/min).

[0099] The separation flow channel may be provided with one or two permanent or electromagnets in three different ways:

(a) Rare earth Samarium-Cobalt block magnets of 1.0 x 1.0 x 0.5 mm (Goudsmit, Netherlands) may be glued with silicon rubber into the opening slot of the separation flow channel.

(b) Rare earth (Sr) magnetic powder (Tropag, Hamburg, Germany) can be mixed with epoxy 1:1 (v/v) and this magnetic paste may be glued into the opening slot of the separation flow channel yielding a thick film magnetic

layer of 1.0 x1.0 x 0.5 mm.

(c) Ferrite steel wool may be glued with silicon rubber into the opening slat of the separation flow channel. A high magnetic field gradient can then be induced inside the opening slots by applying an external magnetic field, e.g. by an electromagnet (Goudsmit, Netherlands) positioned proximate to the separation flow channel.

EXAMPLE 1

[0100] A micro flow system with a layout as sketched in Fig. 5(d) with two inlets and two outlets has been tested utilising it for separation of various magnetisable particles. The test conditions are listed below.

Particle concentration	10^7 particles/ml
Total flow rate	25 μl/min
Length flow chip	3.5 mm
Channel width	250 μm
Channel depth	60 μm
Separation time	2.4 sec
Desired particle deflection:	10 μm

[0101] The separation efficiency (enrichment rate) E and depletion rate 1/E are defined by

$$E=\frac{\dfrac{\text{\% positive particles after separation}}{\text{\% negative particles after separation}}}{\dfrac{\text{\% positive particles before separation}}{\text{\% negative particles before separation}}}$$

[0102] For separation of various paramagnetic standard beads of different sizes and paramagnetic field strength, the results are shown in the Table 2.

Table 2

Separation efficiencies				
Paramagnetic Bead	Size μm	Separation A)	Efficiency B)	[%][1] C)
Polysciences				
25 % iron-oxide	1-10	>99	>99	95
50 % iron-oxide	1-10	>99	>99	96.5
Paesel + Lorel				
Magnetic Affinity	0.5-1.5	>99	>99	97.5
Boehringer				
Streptavidin Magnetic	1	90.5	88.7	89.5
Dynal				
Magnetic Mass Dyal M-450	1-10	98.0	>99	96.5

[1] total flow rates: A)= 10 μl/min, B)= 50 μl/min, C)= 100 μl/min

EXAMPLE 2

[0103] Further, the micro flow system used in Example 1 has also been tested by utilising it for separation of Human T-lymphocytes (JURKAT cells). Magnetically stained and unstained JURKAT cells were used to form a heterogeneous cell sample. For magnetic staining of the cells, a CD4-magnetic surface marker from Miltenyi Biotech was used. JURKAT cells were suspended in 1% PBS/BSA to a concentration of 10^7/ml. Biotin-conjugated CD4 magnetic microbeads were added at 2 μl stock/10^7 cells following the manufacturer instruction.

[0104] The magnetically stained cells (10^7 cells/ml) flowed through the microchip for 10 min. and fluids were collected at the two outlets. Three experiments at different flow rates (5, 25, 50μl/min) were performed. The same experiments were performed using magnetically unstained cells.

[0105] An aliquot of the collected samples was analysed under a microscope and the particles were counted using a Neubauer microscopy chamber. For each experiment 1 µl sample was analysed:

Run	flow rate [µl/min]	cells [%] at Sort outlet
Negative (unstained cells)		
	5	<0.1
	25	<0.1
	50	<0.1
Control[1]		
	5	n.n.
	25	n.n.
	50	n.n.
Positive (stained cells)		
	5	95.5
	25	92.8
	50	80.5
Control[1]		
	5	n.n.
	25	n.n
	50	n.n.

[1] using the micro flow system without an integrated magnet

EXAMPLE 3

[0106] The system employed for separation of magnetisable particles from a sample is shown in Fig. 4. It comprises two syringe infusion pumps (Harvard Apparatus, Southnatik, Az) that provides constant flow rates of 0.1 to 100 µl/min using a 0.5 ml micro syringe (Hamilton, Bonaduz, Switzerland), a separation flow channel of silicon for the separation of the magnetisable particles, and a collecting unit for collecting of the sorted particles. Two 3-way microvalves (Lee, Parameter AB, Sweden) were integrated into the apparatus for sterile solution handling. All components were interconnected with fused silica capillaries (340 µm id., Supelco, U.S.A.). The SFC was placed under an inverted microscope (Axiovert 100, Zeiss, Germany) for visualisation of the separation procedure. All micro channels and tubing were deactivated by silanisation as described in Blankenstem, G. Scampavia L, Branebjerg J, Larsen UD, Ruzicka J (1996): Flow switch for analyte injection and cell/particle sorting in Analytical Methods and Instrumentation, µTAS'96 conference, 17-22 November 1996, Basel. A FACScan with 488 nm argon laser excitation and collection of forward and side scatter and fluorescence of fluorescein were used (Becton Dickinson, Mountain View, CA) for all experiments. Results were collected and analysed using the FACScan research software (Becton Dickinson).

[0107] Results on the use of a separation flow channel equipped with a permanent magnet optimised for Dynal beads are shown in Fig. 10. A bead suspension of 1-5 x 10^8 particles/mi containing a mixture of non labelled magnetic Dynal particles (d: 4.5 µm, M-450) and fluorescence calibration beads (d: 3.2 µm, Dako A/S, Glostrup, Denmark) have been separated. About 1 ml of the non-magnetic, non-deflected fraction was collected at the waste outlet and analysed by flow cytometry (B). To enumerate the positive and negative fractions, two windows were set for the statistic evaluation. Before separation, the sample contained 38.3 % fluorescence particles and 55.8 % magnetic particles, respectively (a). After sorting by the described system almost all magnetic particles were found in the sorted fraction collected from the sort outlet (b) and non-magnetic particles were found in the negative fraction (c) collected from the waste outlet, respectively. Under optimised conditions, an enrichment rate of 350 was achievable.

EXAMPLE 4

[0108] This example concerns enrichment of fetal cells in a sample for magnetic activated cell sorting. A combination of optical cytometry, and Figs. 4 and 8 (lower), magnetic cell separation, provides a powerful apparatus for efficient enrichment of fetal cells in a sample.

[0109] The process for increasing the concentration of fetal cell in maternal blood samples involves the following steps (see Fig. 11): (i) A first selection step for removal of the majority of the maternal blood cells based upon their

volume, size and density; (ii) A second sorting step for isolation of the fetal blood cells from the remaining maternal blood cells based on immuno-fluorescent separation and/or based on immuno-magnetic separation using a device as described in Fig. 4. In the examples shown in Fig. 7(b), the magnetic blood sample is first separated in a magnetic separation chamber, followed by a separation due to optical properties of the sample, or two magnetic separations are performed one after the other, see Fig. 7(c), in order to obtain a highly purified product.

[0110] An example of sorting of particles of very low concentration from a sample of maternal blood in a non-invasive prenatal screening test is presented in the following paragraph.

[0111] Nucleated red blood cells are found in maternal blood in a concentration of 10 to 1000 per ml of all nucleated cells. Bianchi has shown (D.W. Branchi, Journal of Pediatrics, 1995, 127, 6, p. 847-856) that it is possible to use such cells for genetic screening in prenatal diagnosis. The cell surface marker CD71+ for example, is an appropriate marker to select such cells from maternal blood. Test results demonstrates that magnetic activated cell sorting is powerful enrichment system for sorting and isolating fetal nucleated blood cells from maternal blood. For this the magnetic activated cell micro technology as described in this invention is used. Fetal cells are distinguished and separated from maternal blood by the use of a specific surface marker (CD71) which is only present on the cell membrane of fetal nucleated blood cells. By selectively attaching a magnetic antibody probe to CD71, a magnetic probe is attached substantially exclusively to fetal cells.

EXAMPLE 5

[0112] This example concerns depletion of magnetically labelled CD45 positive cells (maternal leukocytes) from a maternal blood sample spiked with cord blood. A flow chip described in Fig. 1 was used in a system as described in Fig: 4. In this experiment a 1:3 spike (fetal/maternal, v/v) was used to demonstrate the performance of the magnetic separation. Heparin was used as an anti-coagulant. The nucleated cells were labelled with CD45 coated magnetic 0.1 μ micro particles (Immunicom, U.S.A.), using a monoclonal antibody against CD45 as the first layer. The cell suspension was collected at both outlets 6 and 7 (see Fig. 1). For testing the sorting efficiency, parts of both the collected fractions were analysed on microscope slides. The results showed that most of the cells, more than 95%, collected at the sort outlet 6 were CD45 positive.

Claims

1. A micro flow system for separating particles, comprising a member having

   a flow channel defined therein for guiding a flow of a fluid containing the particles through the flow channel,

   first inlet means positioned at one end of the flow channel for entering the fluid into the flow channel,

   second inlet means for entering a first guiding buffer for controlling cross-section and flow path through the flow channel of the flow of the fluid containing particles,

   first outlet means positioned at the other end of the flow channel for discharging the fluid from the flow channel,

   the flow of the fluid containing the particles being controlled in such a way that one particle at the time passes a cross-section of the flow channel,

   the member being positioned in a field that is substantially perpendicular to a longitudinal axis of the flow channel so that particles residing in the flow channel and being susceptible to the field across the flow channel are deflected into the first guiding buffer in the direction of the field.

2. A micro flow system according to claim 1, further comprising third inlet means for entering a second guiding buffer, the cross-section and the path through the flow channel of the flow of the fluid containing particles being controlled by the first and second guiding buffer flows that surround the flow of the fluid containing particles.

3. A micro flow system according to claim 2, wherein the width and the position of the flow of fluid containing particles is controlled by adjustment of the volumetric ratio between the fluid flow rate and the flow rate of the guiding buffers.

4. A micro flow system according to any of the preceding claims, wherein the member further comprises field generating means positioned proximate to the flow channel for generating a field substantially perpendicular to a

longitudinal axis of the flow channel.

5. A micro flow system according to any of the preceding claims, further comprising monitoring means positioned at the flow channel for monitoring parameters of a particle residing within a measurement volume within the flow channel and providing an output signal corresponding to a monitored parameter.

6. A micro flow system according to claim 5, wherein the monitoring means comprise optical detection means for monitoring optical parameters of a particle residing within a measurement volume within the flow channel and providing an output signal corresponding to an optical parameter.

7. A micro flow system according to claim 5 or 6, wherein the monitoring means comprise a Hall sensor for measurement of a magnetic parameter of a magnetic particle within a specific volume of the flow channel.

8. A micro flow system according to any of claims 5-7, further comprising field generating control means for controlling the strength of the field generated by the field generating means in response to the output signal of the monitoring means whereby particles are separated according to values of a parameter monitored by the monitoring means.

9. A micro flow system according to any of the preceding claims, wherein the Reynolds number of the flow of the fluid containing the particles through the channel is in the range of 0.01-500, preferably in the range of 0.05-50, in particular in the range of 0.1-25.

10. A micro flow system according to any of claims 1-8, wherein the lowest cross-sectional area of the flow channel is in the range of 0.004-0.11 mm^2.

11. A micro flow system according to any of the preceding claims, further comprising second outlet means for discharging particles having been deflected in the flow channel.

12. A micro flow system according to any of the preceding claims, wherein the field generating means comprises a magnet.

13. A micro flow system according to claim 12, wherein the field generating means further comprise ferrite members positioned at the flow channel for focussing of a magnetic field.

14. A micro flow system according to any of the preceding claims, wherein the field generating means comprises an electrode.

15. A micro flow system according to any of the preceding claims, wherein positions in relation to the flow channel of the field generating means are adjustable for adjustment of the strength of the field across the flow channel.

16. A micro flow system according to any of the preceding claims, further comprising flow speed adjustment means for adjustment of the time the particles reside in the flow channel.

17. A micro flow system according to any of the preceding claims, further comprising a cover for covering the flow channel.

18. A micro flow system according to claim 17, wherein the cover is a transparent or translucent cover allowing optical monitoring of the flow channel.

19. A micro flow system according to any of the preceding claims, wherein the deflected particles comprise living cells.

20. A micro flow system according to any of the preceding claims, wherein the deflected particles comprise beads, microspheres, chromosomes, organelles, biomolecules, or proteins.

21. A micro flow system according to any of the preceding claims, wherein the deflected particles have been magnetically, chromophorically, or fluorescently stained.

22. A micro flow system according to any of the preceding claims, comprising a plurality of outlets for discharging of particles separated according to their different susceptibility to the field across the flow channel.

23. A micro flow system according to any of the preceding claims, wherein the member further comprises pre-treatment and/or post-treatment facilities.

24. A micro flow system according to claim 23, wherein the pre-treatment facilities comprise incubation means for preparing or pre-reacting the fluid comprising the particles.

25. A micro flow system according to claim 23 or 24, wherein the pre-treatment facilities comprise means for magnetic, fluorescent, or chromophoric staining.

26. A micro flow system according to claim 23, wherein the post-treatment facilities comprise means for collecting or concentrating the deflected particles.

27. A micro flow system according to claim 23, wherein the post-treatment facilities comprise means for bringing the deflected particles into contact with one or more reagent(s).

28. A method of separating particles, comprising the steps of

guiding a flow of a fluid containing the particles through a flow channel with a guiding buffer in such a way that one particle at the time passes a cross-section of the flow channel,

positioning the flow channel in a field that is substantially perpendicular to a longitudinal axis of the flow channel so that particles residing in the flow channel and being susceptible to the field across the flow channel are deflected in the direction of the field and thereby separated from the fluid containing particles and into the guiding buffer.

29. A method according to claim 28, wherein the field in which the flow channel is positioned is a magnetic field and the particles comprise fetal cells and maternal cells, and the method comprises the step of

selective magnetically staining of fetal cells so as to make the stained cells susceptible to the field in which the flow channel is positioned.

30. A method according to claim 28, wherein the field in which the flow channel is positioned is a magnetic field and the particles comprise cancer cells and other cells, and the method comprises the step of

selective magnetically staining of cancer cells so as to make the stained cells susceptible to the field in which the flow channel is positioned.

Patentansprüche

1. Mikrodurchfluss-System zur Trennung von Partikeln, umfassend ein Mitglied mit

einem hier definierten Durchflusskanal, der den Fluss einer Partikel enthaltenden Flüssigkeit durch den Durchflusskanal leitet,

ersten Einlassmitteln, die sich an einem Ende des Durchflusskanals befinden zum Einführen der Flüssigkeit in den Durchflusskanal,

zweiten Einlassmitteln zum Einführen eines ersten leitenden Puffers zur Steuerung des Querschnitts und des Flusses der Partikel enthaltenden Flüssigkeit den Flussweg entlang durch den Durchflusskanal,

erste Auslassmittel, die sich am anderen Ende des Durchflusskanals befinden, zum Abführen der Flüssigkeit aus dem Durchflusskanal,

wobei der Fluss der Partikel enthaltenden Flüssigkeit auf eine solche Art gesteuert wird, dass ein Partikel nach dem anderen einen Querschnitt des Durchflusskanals passiert,

wobei das Mitglied in einem Feld, das im wesentlichen senkrecht zur Längsachse des Durchflusskanals ver-

läuft, so positioniert ist, dass die Partikel, die sich im Durchflusskanal befinden, und die durch das Feld über den Durchflusskanal hinweg beeinflussbar sind, in den ersten leitenden Puffer in Richtung des Feldes abgelenkt werden.

2. Mikrodurchfluss-System nach Anspruch 1, das außerdem dritte Einlassmittel zum Einführen eines zweiten Puffers umfasst, wobei der Querschnitt und der Flussweg der Partikel enthaltenden Flüssigkeit durch den Fluss des ersten und zweiten leitenden Puffers gesteuert werden, die den Fluss der Partikel enthaltenden Flüssigkeit umgeben.

3. Mikrodurchfluss-System nach Anspruch 2, wobei die Breite und die Position des Flusses der Partikel enthaltenden Flüssigkeit durch Einstellen des volumetrischen Verhältnisses zwischen der Flüssigkeitsfließrate und der Fließrate der leitenden Puffer gesteuert wird.

4. Mikrodurchfluss-System nach einem der vorhergehenden Ansprüche, wobei das Mitglied außerdem felderzeugende Mittel umfasst, die sich in der Nähe des Durchflusskanals befinden, zum Erzeugen eines Feldes, das im wesentlichen senkrecht zur Längsachse des Durchflusskanals verläuft.

5. Mikrodurchfluss-System nach einem der vorhergehenden Ansprüche außerdem umfassend Nachweismittel, die sich beim Durchflusskanal befinden, zum Nachweisen von Parametern eines Partikels, der sich im Messvolumen in dem Durchflusskanal befindet und ein Ausgangssignal liefert, das einem nachgewiesenen Parameter entspricht.

6. Mikrodurchfluss-System nach Anspruch 5, wobei die Nachweismittel optische Nachweismittel umfassen zum Nachweisen von optischen Parametern eines Partikels, der sich im Messvolumen in dem Durchflusskanal befindet und ein Ausgangssignal liefert, das einem optischen Parameter entspricht.

7. Mikrodurchfluss-System nach Anspruch 5 oder 6, wobei die Nachweismittel einen Hallsensor umfassen zum Messen eines magnetischen Parameters eines magnetischen Partikels in einem spezifischen Volumen des Durchflusskanals.

8. Mikrodurchfluss-System nach einem der Ansprüche 5 bis 7, außerdem umfassend felderzeugende Kontrollmittel zum Kontrollieren der Feldstärke, die durch die felderzeugenden Mittel erzeugt wird als Folge des Ausgangssignals der Nachweismittel, wobei Partikel gemäß der Parameterwerte, die durch die Nachweismittel nachgewiesen werden, getrennt werden.

9. Mikrodurchfluss-System nach einem der vorhergehenden Ansprüche, wobei die Reynoldszahl des Flusses der Partikel enthaltenden Flüssigkeit durch den Kanal im Bereich zwischen 0,01 - 500, bevorzugt im Bereich von 0,05 - 50, insbesondere im Bereich von 0,1 - 25 liegt.

10. Mikrodurchfluss-System nach einem der Ansprüche 1 - 8, wobei die niedrigste Querschnittsfläche des Durchflusskanals im Bereich von 0,004 - 0,11 mm^2 liegt.

11. Mikrodurchfluss-System nach einem der vorhergehenden Ansprüche, außerdem umfassend zweite Auslassmittel zum Abführen von Partikeln, die im Durchflusskanal abgelenkt wurden.

12. Mikrodurchfluss-System nach einem der vorhergehenden Ansprüche, wobei die felderzeugenden Mittel einen Magnet umfassen.

13. Mikrodurchfluss-System nach Anspruch 12, wobei die felderzeugenden Mittel außerdem Ferritmitglieder umfassen, die sich beim Durchflusskanal befinden, zum Fokussieren eines magnetischen Feldes.

14. Mikrodurchfluss-System nach einem der vorhergehenden Ansprüche, wobei die felderzeugenden Mittel eine Elektrode umfassen.

15. Mikrodurchfluss-System nach einem der vorhergehenden Ansprüche, wobei die Positionen der felderzeugenden Mittel im Verhältnis zum Durchflusskanal einstellbar sind zum Einstellen der Feldstärke über den Durchflusskanal hinweg.

16. Mikrodurchfluss-System nach einem der vorhergehenden Ansprüche, außerdem umfassend Fließgeschwindigkeitseinstellende Mittel zum Einstellen der Zeit, in der sich die Partikel in dem Durchflusskanal befinden.

17. Mikrodurchfluss-System nach einem der vorhergehenden Ansprüche, außerdem umfassend eine Abdeckung zum Abdecken des Durchflusskanals.

18. Mikrodurchfluss-System nach Anspruch 17, wobei die Abdeckung eine transparente oder translucente Abdeckung ist, durch die optische der Durchflusskanal beobachtet werden kann.

19. Mikrodurchfluss-System nach einem der vorhergehenden Ansprüche, wobei die abgelenkten Partikel lebende Zellen umfassen.

20. Mikrodurchfluss-System nach einem der vorhergehenden Ansprüche, wobei die abgelenkten Partikel Kügelchen, Mikrosphären, Chromosomen, Organellen, Biomoleküle oder Proteine umfassen.

21. Mikrodurchfluss-System nach einem der vorhergehenden Ansprüche, wobei die abgelenkten Partikel magnetisch, chromophor oder fluoreszent gefärbt worden sind.

22. Mikrodurchfluss-System nach einem der vorhergehenden Ansprüche, umfassend eine Vielzahl von Auslassen zum Abführen der Partikel, die über den Durchflusskanal hinweg gemäß ihrer unterschiedlichen Beeinflussbarkeit durch das Feld getrennt werden.

23. Mikrodurchfluss-System nach einem der vorhergehenden Ansprüche, wobei das Mitglied außerdem Vorbehandlungsund/oder Nachbehandlungsmöglichkeiten umfasst.

24. Mikrodurchfluss-System nach Anspruch 23, wobei die Vorbehandlungsmöglichkeiten Inkubationsmittel umfassen zum Herstellen oder Vorreagieren der die Partikel enthaltenden Flüssigkeit umfasst.

25. Mikrodurchfluss-System nach Anspruch 23 oder 24, wobei die Vorbehandlungsmöglichkeiten Mittel umfassen zur magnetischen, fluoreszierenden oder chromophoren Färbung.

26. Mikrodurchfluss-System nach Anspruch 23, wobei die Nachbehandlungsmöglichkeiten Mittel umfassen zum Sammeln und Konzentrieren der abgelenkten Partikel.

27. Mikrodurchfluss-System nach Anspruch 23, wobei die Nachbehandlungsmöglichkeiten Mittel umfassen, um die abgelenkten Partikel in Kontakt mit einem oder mehreren Reagenzien zu bringen.

28. Verfahren zum Trennen von Partikeln, umfassend die Schritte:

Leiten des Flusses einer Partikel enthaltenden Flüssigkeit durch einen Durchflusskanal mit einem leitenden Puffer auf eine solche Weise, dass ein Partikel nach dem anderen einen Querschnitt des Durchflusskanals passiert,
Positionieren des Durchflusskanals in einem Feld, das im wesentlichen senkrecht zur Längsachse des Durchflusskanals verläuft, so dass Partikel, die sich im Durchflusskanal befinden und die durch das Feld über den Durchflusskanal hinweg beeinflussbar sind, in die Richtung des Feldes abgelenkt werden und dadurch von der die Partikel enthaltenden Flüssigkeit in den leitenden Puffer getrennt werden.

29. Verfahren nach Anspruch 28, wobei das Feld, in dem der Durchflusskanal positioniert ist, ein magnetisches Feld ist, und die Partikel fetale und maternale Zellen umfassen und das Verfahren die Schritte umfasst:

selektives magnetisches Färben der fetalen Zellen, so dass die gefärbten Zellen durch das Feld, in dem der Durchflusskanal positioniert ist, beeinflussbar sind.

30. Verfahren nach Anspruch 28, wobei das Feld, in dem der Durchflusskanal positioniert ist, ein magnetisches Feld ist, und die Partikel Krebszellen und andere Zellen umfassen und das Verfahren den folgenden Schritt umfasst:

selektives magnetisches Färben der Krebszellen, um die gefärbten Zellen für das Feld, in dem der Durchflusskanal positioniert ist, beeinflussbar zu machen.

Revendications

1. Un système à microdébit pour séparer des particules qui comprend un organe incluant:

   un canal de flux qui y est défini pour guider un flux d'un fluide contenant les particules à travers le canal de flux, un premier moyen d'entrée positionné à une première extrémité du canal de flux pour introduire le flux dans le canal de flux;
   un deuxième moyen d'entrée pour introduire un premier tampon de guidage pour régler une section transversale et un trajet de flux, à travers le canal de flux, de flux du flux de fluide qui contient les particules,
   un premier moyen de sortie positionné à l'autre extrémité du canal de flux pour décharger le flux à partir du canal de fluide,
   le flux du fluide contenant les particules étant réglé d'une manière telle que les particules passent une à la fois à une section transversale du canal de flux;
   l'organe étant positionné dans un champ qui est sensiblement perpendiculaire à un axe longitudinal du canal de flux d'une manière telle que des particules qui résident dans le canal de flux et sont sensibles au champ transversal au canal de flux sont défléchies pour entrer dans le premier tampon de guidage dans la direction du champ.

2. Un système à microdébit selon la revendication 1, qui comprend en outre un troisième moyen d'entrée pour introduire un deuxième tampon de guidage, la section transversale et le trajet, à travers le canal de flux, du flux du fluide qui contient les particules étant réglés par les flux du premier et du deuxième tampons de guidage qui entourent le flux du fluide qui contient les particules.

3. Un système à microdébit selon la revendication 2, dans lequel la largeur et la position du flux de fluide qui contient les particules sont réglés par ajustement du rapport volumétrique entre le débit de fluide et le débit des tampons de guidage.

4. Un système à microdébit selon l'une quelconque des revendications précédentes, dans lequel l'organe comprend en outre un moyen générateur de champ positionné à proximité du canal de flux pour engendrer un champ sensiblement perpendiculaire à un axe longitudinal du canal de flux.

5. Un système à microdébit selon l'une quelconque des revendications précédentes, qui comprend en outre un moyen de contrôle positionné au canal de flux pour contrôler des paramètres d'une particule qui réside à l'intérieur d'un volume de mesure à l'intérieur du canal de flux et envoyer un signal de sortie qui correspond à un paramètre contrôlé.

6. Un système à microdébit selon la revendication 5, dans lequel le moyen de contrôle comprend un moyen de détection optique pour contrôler des paramètres optiques d'une particule résidant à l'intérieur d'un volume de mesure à l'intérieur du canal de flux et envoyer un signal de sortie qui correspond à un paramètre optique.

7. Un système à microdébit selon la revendication 5 ou 6, dans laquelle le moyen de contrôle comprend un capteur de Hall pour mesurer un paramètre magnétique d'une particule magnétique à l'intérieur d'un volume spécifique du canal de flux.

8. Un système à microdébit selon l'une quelconque des revendications 5 à 7, qui comprend en outre un moyen de réglage de génération de champ pour régler l'intensité du champ engendré par le moyen générateur de champ en réponse au signal de sortie du moyen de contrôle d'une manière telle que des particules sont séparées en fonction de valeurs d'un paramètre contrôlé par le moyen de contrôle.

9. Un système à microdébit selon l'une quelconque des revendications précédentes, dans lequel le nombre de Reynolds du flux du fluide qui contient les particules à travers le canal est dans la plage de 0,01 à 500, de préférence dans la plage de 0,05 à 50, en particulier dans la plage de 0,1 à 25.

10. Un système à microdébit selon l'une quelconque des revendications 1 à 8, dans lequel la plus petite superficie, en section transversale, du canal de flux est dans la plage de 0,004 à 0,11 mm^2.

11. Un système à microdébit selon l'une quelconque des revendications précédentes, qui comprend en outre un deuxième moyen de sortie pour décharger des particules qui ont été défléchies dans le canal de flux.

12. Un système à microdébit selon l'une quelconque des revendications précédentes, dans lequel le moyen générateur de champ comprend un aimant.

13. Un système à microdébit selon la revendication 12, dans lequel le moyen générateur de champ comprend en outre des organes en ferrite positionnés au canal de flux pour focaliser un champ magnétique.

14. Un système à microdébit selon l'une quelconque des revendications précédentes, dans lequel le moyen générateur de champ comprend une électrode.

15. Un système à microdébit selon l'une quelconque des revendications précédentes, dans lequel des positions du moyen générateur de champ, par rapport au canal de flux, peuvent être ajustées pour ajuster l'intensité du champ transversalement au canal de flux.

16. Un système à microdébit selon l'une quelconque des revendications précédentes, qui comprend en outre un moyen d'ajustement de la vitesse du flux pour ajuster le temps pendant lequel les particules résident dans le canal de flux.

17. Un système à microdébit selon l'une quelconque des revendications précédentes, qui comprend en outre un couvercle pour couvrir le canal de flux.

18. Un système à microdébit selon la revendication 17, dans lequel le couvercle est un couvercle transparent ou translucide qui permet un contrôle optique du canal de flux.

19. Un système à microdébit selon l'une quelconque des revendications précédentes, dans lequel les particules défléchies comprennent des cellules vivantes.

20. Un système à microdébit selon l'une quelconque des revendications précédentes, dans lequel les particules défléchies comprennent des perles, des microsphères, des chromosomes, des organelles, des biomolécules ou des protéines.

21. Un système à microdébit selon l'une quelconque des revendications précédentes, dans lequel les particules défléchies ont été colorées de manière magnétique, chromophore ou fluorescente.

22. Un système à microdébit selon l'une quelconque des revendications précédentes, qui comprend une série de sorties pour décharger des particules séparées en fonction de leur sensibilité différente au champ transversal au canal de flux.

23. Un système à microdébit selon l'une quelconque des revendications précédentes, dans lequel l'organe comprend en outre des installations de traitement préalable et de traitement postérieur.

24. Un système à microdébit selon la revendication 23, dans lequel les installations de traitement préalable comprennent un moyen d'incubation destiné à une préparation ou à une réaction préalable du fluide comprenant les particules.

25. Un système à microdébit selon la revendication 23 ou 24, dans lequel les installations de traitement préalable comprennent un moyen de coloration magnétique, fluorescente ou chromophore.

26. Un système à microdébit selon la revendication 23, dans lequel les installations de traitement postérieur comprennent un moyen de collecte ou de concentration des particules défléchies.

27. Un système à microdébit selon la revendication 23, dans lequel les installations de traitement postérieur comprennent un moyen qui amène les particules défléchies au contact d'un ou plusieurs réactif(s).

28. Un procédé de séparation de particules comprenant les étapes consistant à:

guider un flux d'un fluide contenant les particules à travers un canal de flux au moyen d'un tampon de guidage de façon que les particules passent une à la fois à une section transversale du canal de flux,
positionner le canal de flux dans un champ qui est sensiblement perpendiculaire à un axe longitudinal du canal de flux d'une manière telle que les particules qui résident dans le canal de flux et sont sensibles au champ

transversal au canal de flux sont défléchies dans la direction du champ et sont ainsi séparées du fluide contenant les particules et entrent dans le tampon de guidage.

29. Un procédé selon la revendication 28, dans lequel le champ où le canal de flux est positionné est un champ magnétique et les particules comprennent des cellules foetales et des cellules maternelles, et le procédé comprend les étapes consistant à:

colorer magnétiquement les cellules foetales sélectivement afin de rendre les cellules colorées sensibles au champ dans lequel le canal de flux est positionné.

30. Un procédé selon la revendication 28, dans lequel le champ dans lequel le canal de flux est positionné est un champ magnétique et les particules comprennent des cellules cancéreuses et d'autres cellules, et le procédé comprend l'étape consistant à:

colorer magnétiquement des cellules cancéreuses sélectivement afin de rendre les cellules teintées sensibles au champ dans lequel le canal de flux est positionné.

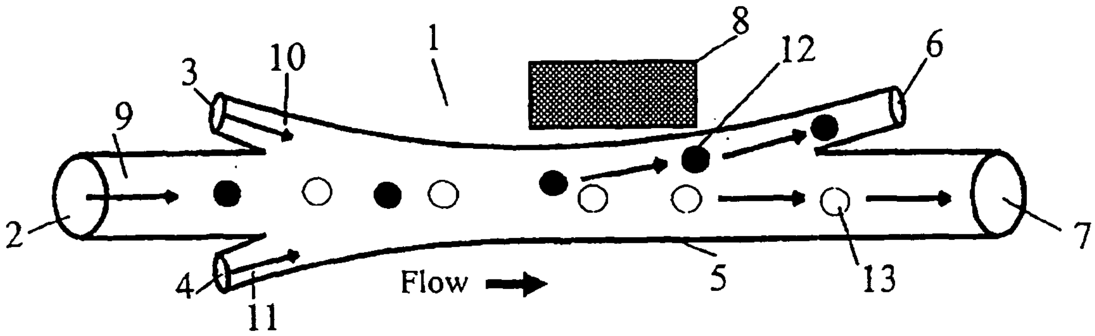

8
1
10
12
6
3
9
2
7
4
11
Flow
5
13

Fig. 1

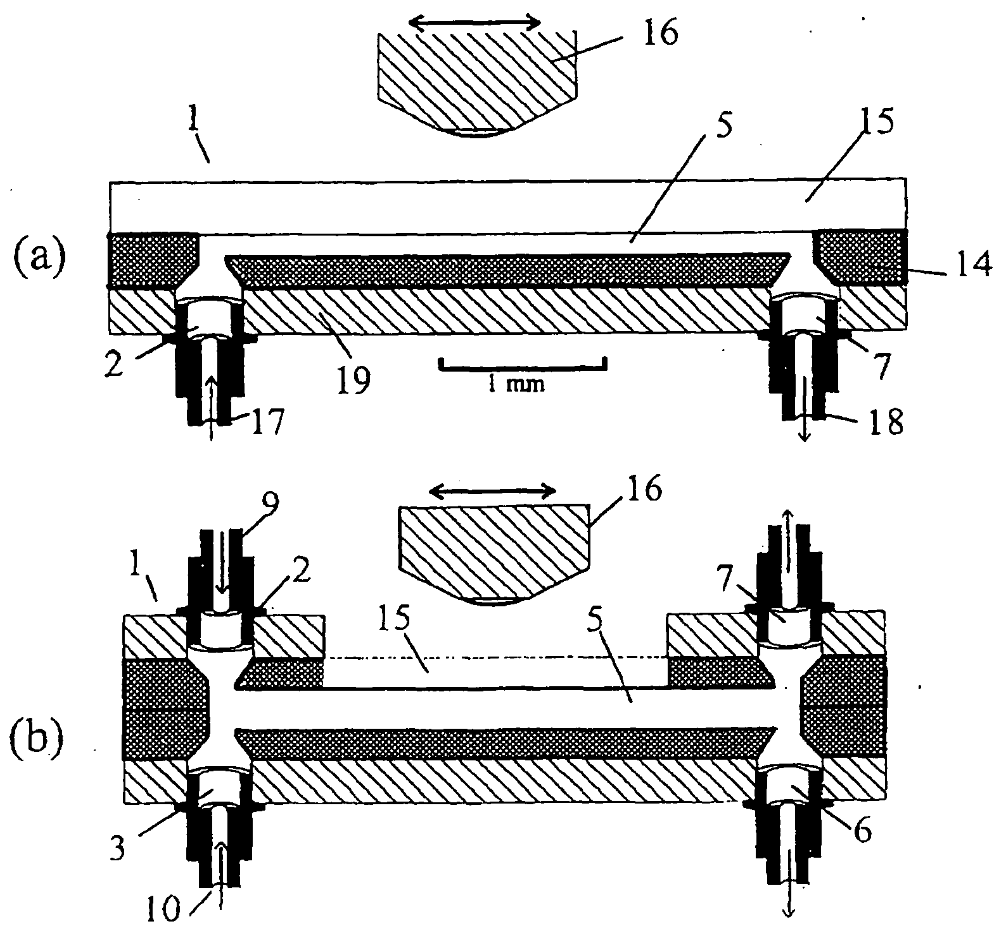

16
1
5
15
(a)
14
2
19
1 mm
7
17
18
16
9
1
2
7
15
5
(b)
3
6
10

Fig. 2

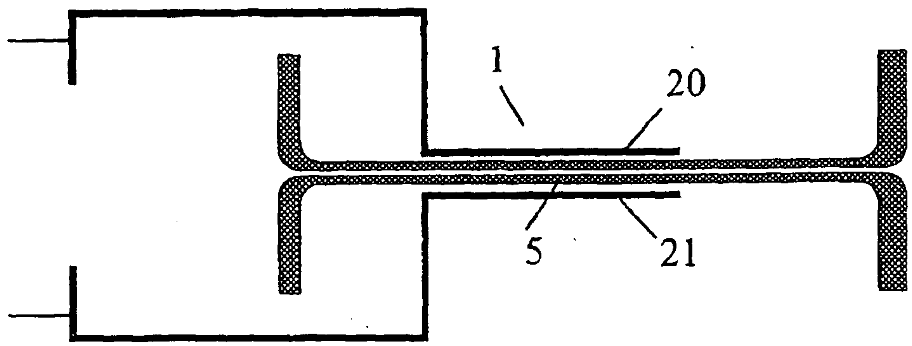


Fig. 3

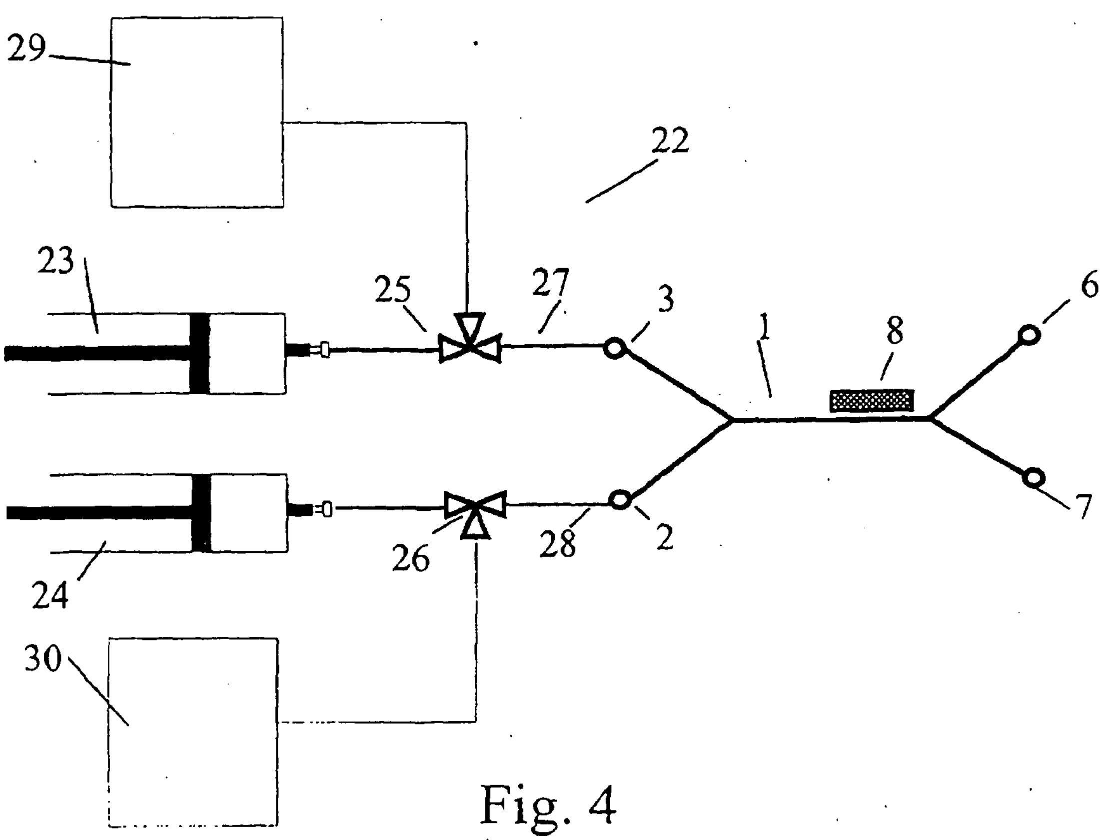


Fig. 4

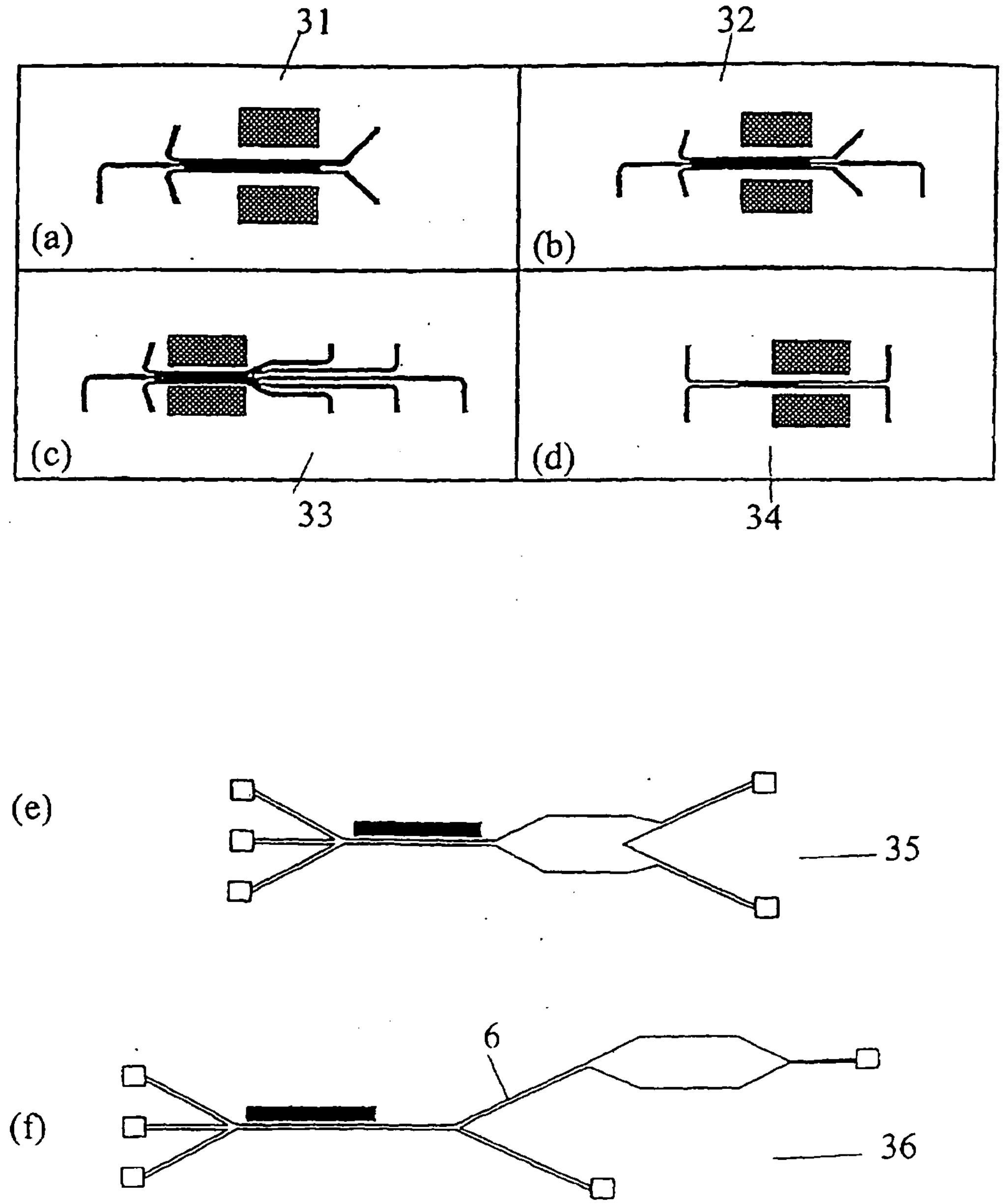


Fig. 5

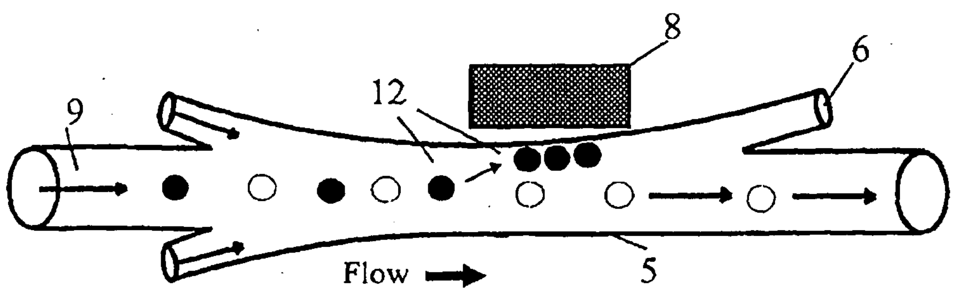


Fig. 6

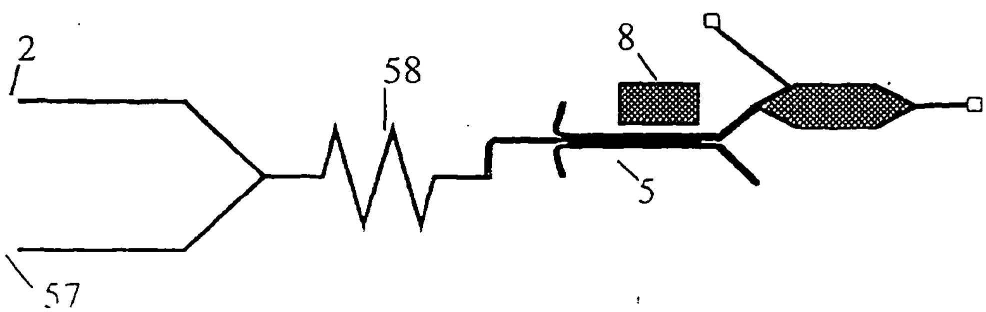
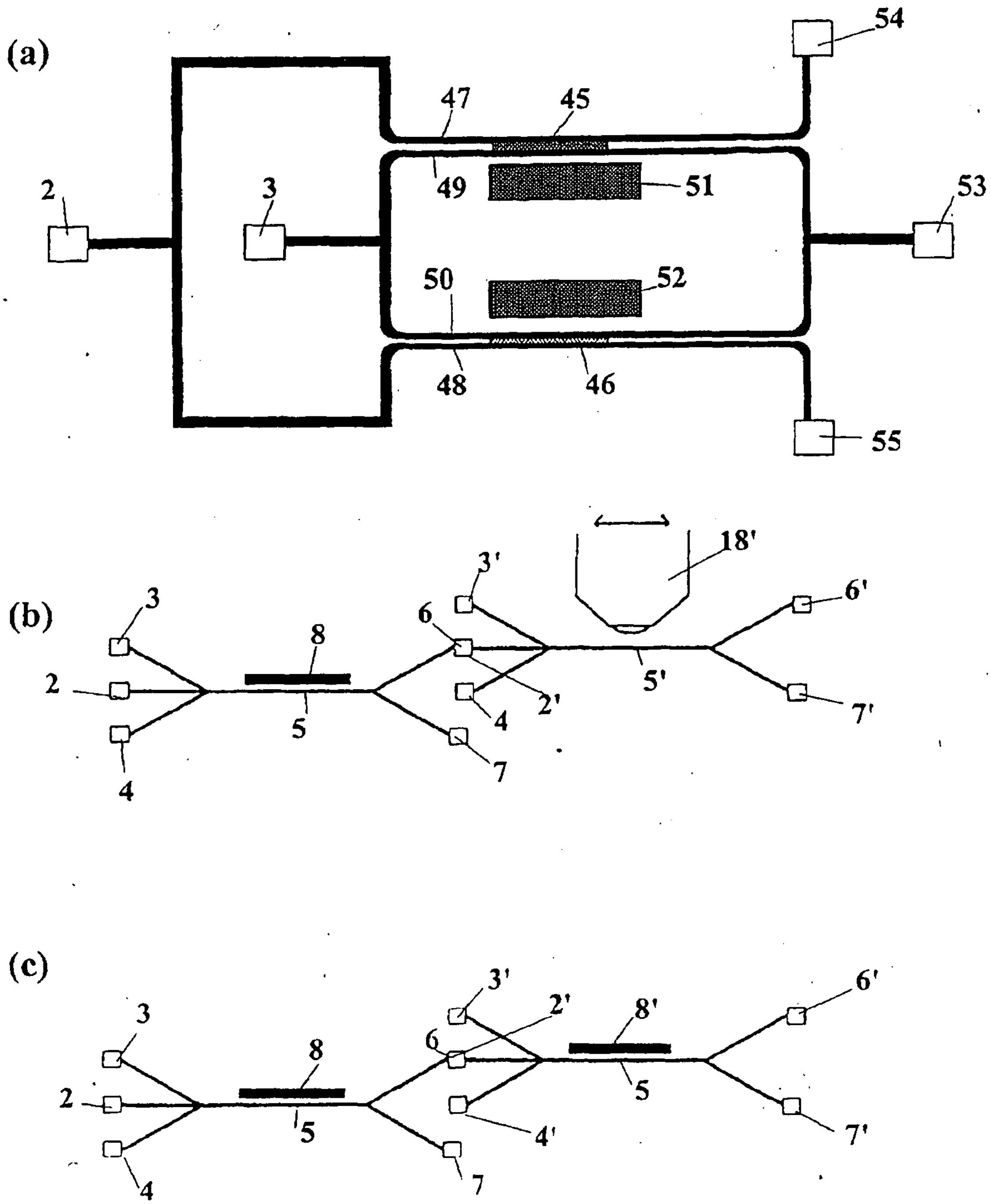


Fig. 8


(a)
54
47
45
2
3
49
51
53
50
52
48
46
55
(b)
18'
3'
6'
3
6
8
2
2'
5'
5
4
7'
4
7
(c)
3'
6'
2'
8'
3
6
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2
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5
4'
7'
4
7

Fig. 7

1)

2)

3)

4)

5)

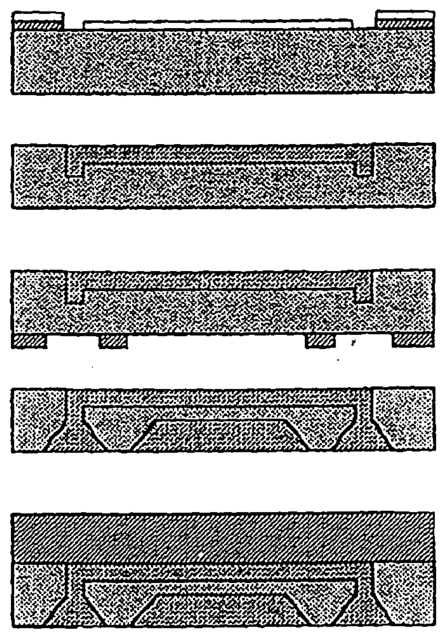

Fig. 9

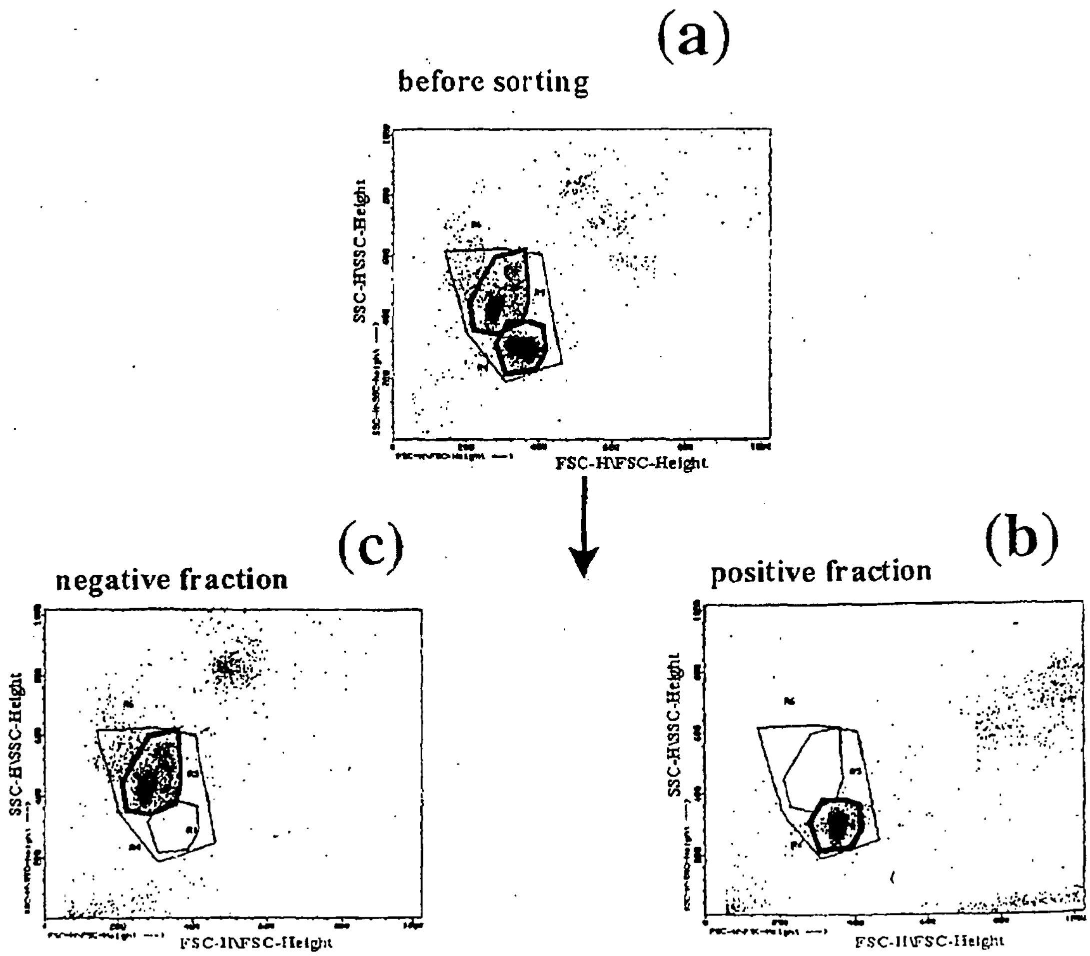
(a)
before sorting
SSC-H\SSC-Height
FSC-H\FSC-Height
(c)
negative fraction
SSC-H\SSC-Height
FSC-H\FSC-Height
(b)
positive fraction
SSC-H\SSC-Height
FSC-H\FSC-Height

Fig. 10

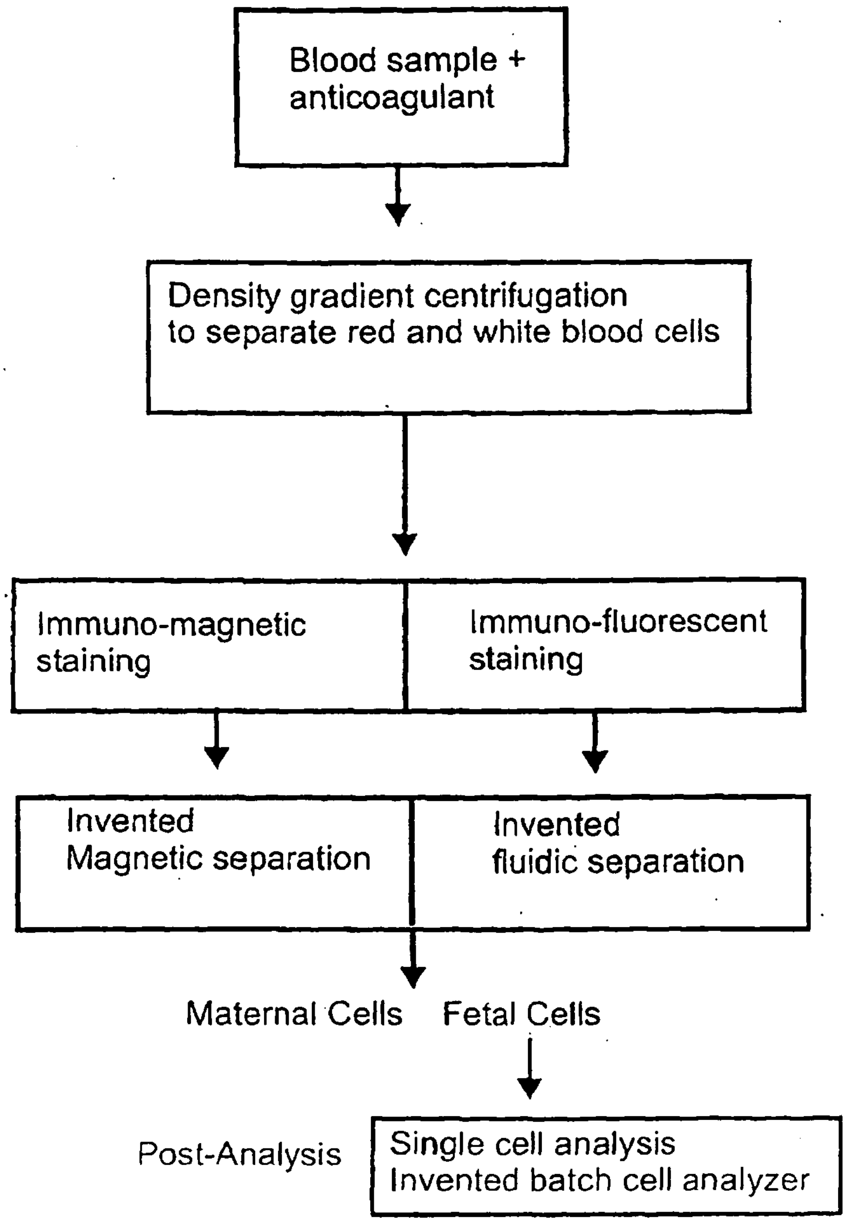
Blood sample + anticoagulant
Density gradient centrifugation to separate red and white blood cells
Immuno-magnetic staining
Immuno-fluorescent staining
Invented Magnetic separation
Invented fluidic separation
Maternal Cells
Fetal Cells
Post-Analysis
Single cell analysis
Invented batch cell analyzer

Fig. 11